Coxal Organs of Chilopoda: the Exocrine Glands in *Lithobius forficatus*

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ABSTRACT

The exocrine glands within the coxal organs of *Lithobius forficatus* are described. Each gland consists of secretory cells, an additional cell and a canal cell, forming a cuticular ductule (class 3 gland according to NOIROT & QUENNEDY, 1974). Secretory cells are rich in rER and PA-TCH-SP (periodic acid-thiocarbohydrazide-silver proteinate)-positive secretory granules. The additional or intercalary cells possess numerous mitochondria and prominent infoldings of the plasma membrane beneath the cuticle of the transport duct. PA-TCH-SP-positive secretory products are obviously discharged along the cuticular ductule into the pore channel of the coxal organ, forming a mucous layer that covers the specialized cuticle of the transport epithelium. Within its subcuticle, chloride can be localized cytochemically; its accumulation in the mucous layer is not significant.

RÉSUMÉ

Organes coxaux des Chilopodes : les glandes exocrines de *Lithobius forficatus*.

Les glandes exocrines des organes coxaux de *Lithobius forficatus* sont décrites. Chaque glande est composée de cellules sécrétrices, d'une cellule additionnelle et d'une cellule-canal, constituant un canalicule cuticulaire [glande de classe 3 selon NOIROT & QUENNEDY (1974)]. Les cellules sécrétrices sont riches en rER et en PA-TCH-SP ("periodic acid-thiocarbohydrazide-silver proteinate") sous forme de granules de sécrétion. Les cellules additionnelles ou intercalaires possèdent de nombreuses mitochondries et des protubérances en doigts de gant de la membrane du plasma en dessous de la cuticule du canalicule. Les produits sécrétés (PA-TCH-SP-positif) sont évacués le long du conduit cuticulaire jusqu'au pore de l'organe coxal, formant une couche de mucus qui couvre la cuticule spécialisée de l'épithélium de transport. A l'intérieur de sa sous-cuticule, les chlorures peuvent être localisés cytochimiquement ; leur accumulation dans la couche de mucus n'est pas significative.

INTRODUCTION

Coxal organs of Chilopoda are complex and possibly multifunctional structures. They are localized on the coxae of the last trunk segment (Geophilomorpha, Scolopendromorpha) or last four trunk segments (Lithobiomorpha) and characterized by numerous pores, each leading into a cuticle-lined pore channel surrounded by a columnar single-layered transport epithelium and - arranged like a collar - junctional cells and several exocrine glands pouring out their secretory products into the lumen of the pore channel. This secretion covers the specialized cuticle of the transport epithelium (for review see ROSENBERG, 1985).

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The general ultrastructure of the coxal organs in Chilopoda (ROSENBERG, 1982, 1983a, b, 1984, 1990) and also in experimental studies, particularly those on *Lithobius forficatus*, suggest that they are involved in water vapour uptake from the environment (ROSENBERG, 1985; ROSENBERG & BAJORAT, 1984). More recently, some evidence has been accumulated suggesting that coxal organs of Lithobiomorpha release a sex-specific pheromone (LITTLEWOOD, 1988, 1991; LITTLEWOOD & BLOWER, 1987). The authors speculate that sub-epithelial blood cells beneath the coxal organs might synthesize this pheromone, which moves across the epithelium of the coxal organ. These assumptions prompted us to examine the "glandular system" of the coxal organs, especially as recent investigations have revealed numerous small epidermal glands close to the coxal pores (ROSENBERG, 1994). On principle such structures could act as pheromone glands.

The following note deals with the exocrine glands of the coxal organs in *Lithobius forficatus* which were not described by LITTLEWOOD (1983) in Lithobiomorpha.

MATERIALS AND METHODS

Coxae of adult *Lithobius forficatus* were fixed as described previously (ROSENBERG 1983a).

For detecting "mucosubstances", animals were fixed with 2.5% glutar dialdehyde and 2% formaldehyde (freshly prepared from paraformaldehyde) in phosphate buffer without any postfixation. After graded ethanol dehydration, tissue was embedded in LR White (London Resin Co.). Ultrathin sections were mounted on formvar-coated Ni grids. The periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-SP) reaction was performed as described by NEISS (1988). The sections remained unstained.

For demonstration of chloride the coxae were fixed according to WICHARD & KOMNICK (1973) with osmium tetroxide and silver lactate and treated with nitric acid during dehydration.

Sections were examined in a Zeiss 109 T electron microscope.

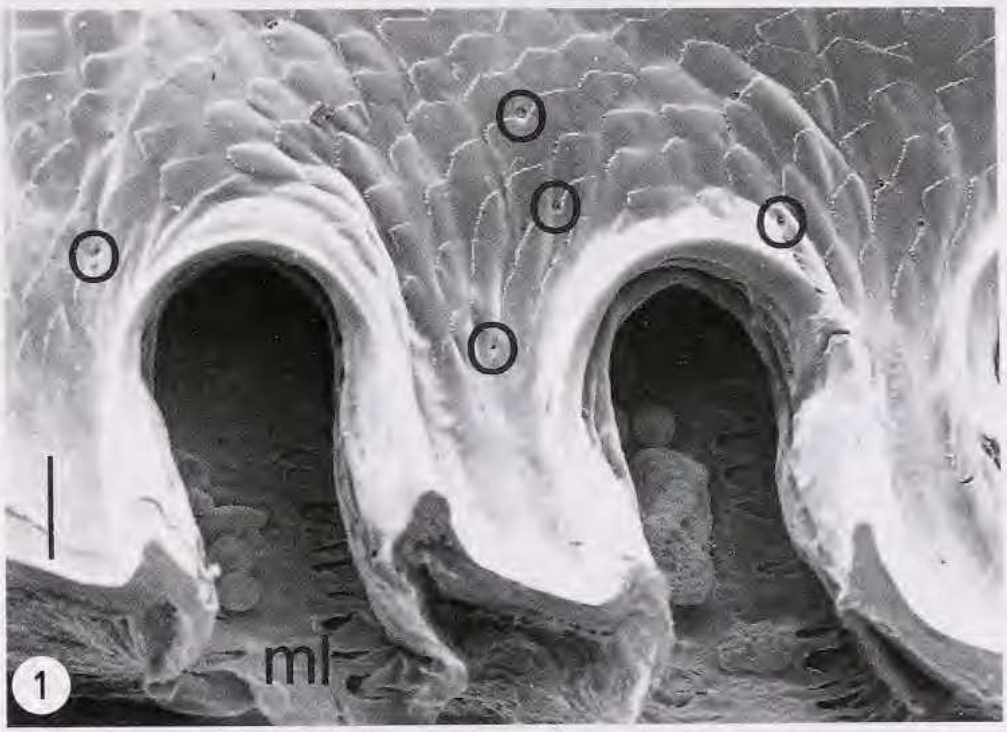
RESULTS

The topography of the exocrine glands within the coxal organs of *Lithobius forficatus* has been described elsewhere (ROSENBERG, 1983a, 1985). Each exocrine gland consists of three types of cells: secretory cells with well-developed granular ER, an additional or intercalary cell, forming microvilli-like projections surrounding a cuticular ductule, and a canal cell, whose cuticular duct runs into the pore channel (Figs 1 & 5).

The secretory cells are spheroidal or spindle-shaped. Their plasma membrane is moderately infolded. Cells are connected with adjacent additional cells by septate desmosomes. The cytoplasm of most secretory cells is packed with stacks of granular endoplasmic reticulum, which is filled with a fineley particulate substance (Fig. 3). In addition, these cells contain numerous dictyosomes in different stages of development and numerous electron dense secretory granules, which are membrane bounded and vary widely in size. Small mitochondria, and some lysosomes and multivesicular bodies, are distributed randomly throughout the cytoplasm. Each secretory cell has a large, lobate nucleus; its chromatin is mostly located peripherally. The thin cuticular ductule of the secretory cell is continuous with the wall of the transport duct of the additional cell.

PA-TCH-SP reaction reveals precipitations at the margins of secretory granules and in some profiles, derived from endoplasmic reticulum (Fig. 6).

The additional duct forming cell is more elongated and its cytoplasm is lighter than that of the secretory cells or adjacent cells of the transport epithelium. Large profiles of endoplasmic reticulum and dictyosomes are absent, and the small nucleus is oval. The plasma membrane surrounding the cuticular duct is folded forming long microvilli-like projections (Fig. 4). Mitochondria are numerous along the apical infoldings, they are larger than in the secretory cell. The wall of the cuticular duct is continuous with the cuticle of the small canal cell and that of the epicuticle of the pore channel of the coxal organ (Fig. 5). As seen by SEM (Fig. 2) and TEM (Fig. 1), secretion is deposited as a distinct mucous layer on the cuticle of the transport epithelium.



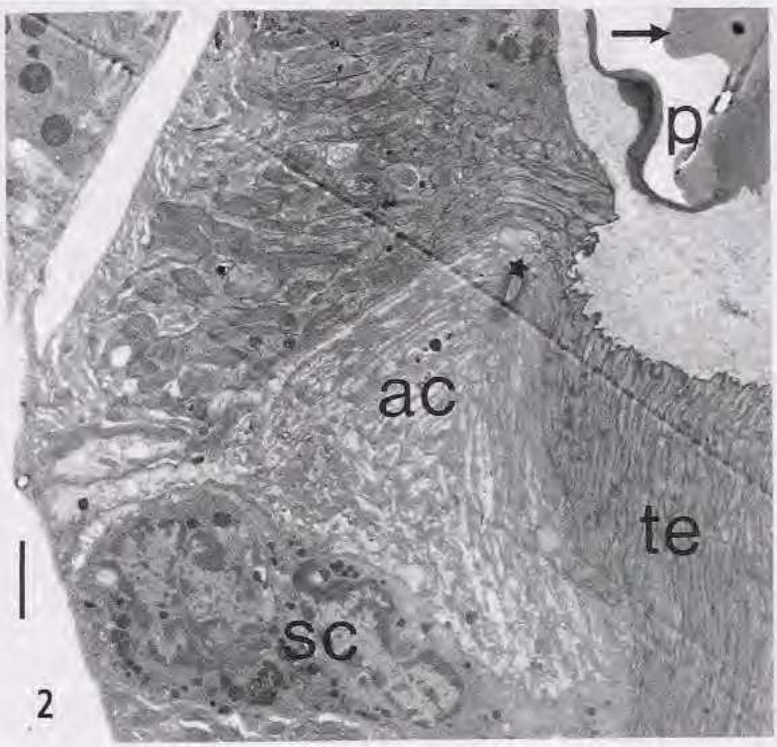


FIG. 1. — Section of two coxal pores of *Lithobius forficatus*, showing the mucous layer (ml) on the bottom of the pore channel and several openings of epidermal glands (circles) around the coxal pores. Scale line, 20 µm.

FIG. 2. — Part of the coxal organ of *Lithobius forficatus* with the transport epithelium (te) and its specialized cuticle, covered by the mucous layer (arrow), and the exocrine gland with secretory cells (sc) and an additional cell (ac) with its cuticular duct (*). p pore channel. Scale line, 0.08 µm.

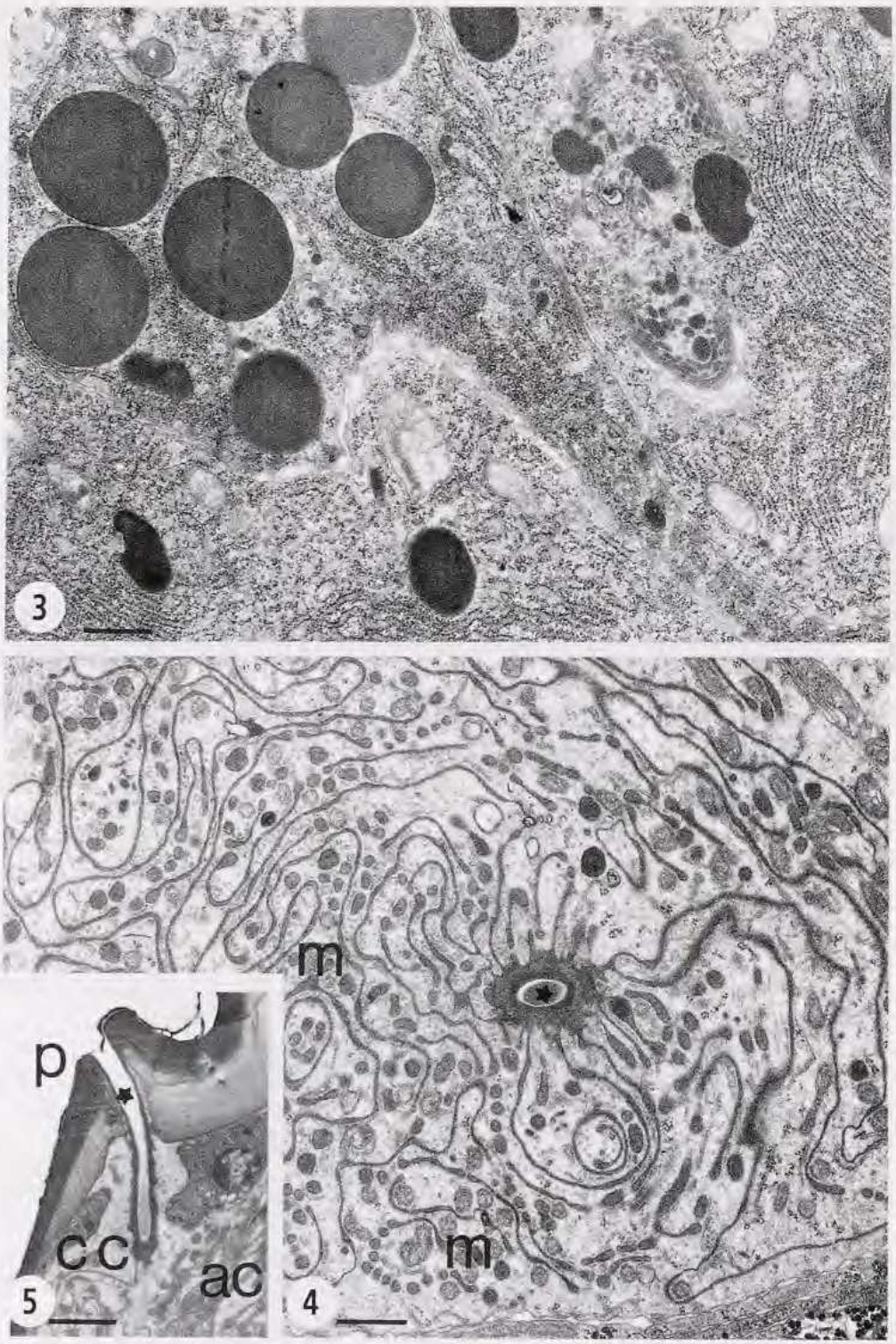


FIG. 3. — Secretory cells of the exocrine gland of the coxal organ with stacks of ER, dictyosomes, and secretory products. Scale line, 0.08 µm.

FIG. 4. — Additional cell of the exocrine gland of the coxal organ. The cuticular ductule (*) is surrounded by infoldings of the apical plasma membrane. m: mitochondrium. Scale line, 0.08 µm.

FIG. 5. — Additional cell (ac) and canal cell (cc) of the exocrine gland of the coxal organ. Their cuticular ductule (*) opens into the pore channel (p) of the coxal organ. Scale line, 0.1 µm.

Within the accessory cells (intercalary and canal cell), treatment by PA-TCH-SP stains the content of the transport duct, its cuticle, the material underlying the cuticle of the duct and the space between the microvilli-like projections (Fig. 7). A positive reaction is also seen in the mucous layer, covering the modified cuticle of the main epithelium (Fig. 8).

In coxal organs, fixed in the osmium-silver-lactate mixture, coarse precipitates are localized predominantly in the subcuticle of the specialized cuticle, covering the transport epithelium (Fig. 9). Fine precipitations seem to be scattered within the overlying endocuticle and in the mucous layer (Fig. 10). No precipitates are found within the cells of the transport epithelium, within the cuticle of the pore channel (Fig. 9), or within the cells of the exocrine gland.

DISCUSSION

The exocrine glands described for the coxal organs of *Lithobius forficatus* can be characterized as "class 3 glands" according to NOIROT & QUENNEDY (1974). In a simple case "a cuticular ductule or canal penetrates the gland cell and the canal runs into a ductule or canal cell which has secreted it" (p. 63). Within the exocrine glands of *Lithobius forficatus*, this description is complicated by the presence of an additional or intercalary cell between the two others. In *Lithobius forficatus* "class 3 glands" appear to be common; they are known to be present adjacent to the telopodal glands and are associated with sensilla trichodea (KEIL, 1975) as well as with the organs of Tömösvary (TICHY, 1973). It is assumed that these glands, although similar in organization, produce substances of different chemical composition and significance.

Considering the ultrastructure of secretory cells, in particular the abundant rough endoplasmic reticulum and the electron dense granules, there is reason to believe that secretory products contain a considerable amount of proteins. However, well-developed dictyosomes and the positive reaction after PA-TCH-SP are indicative of a carbohydrate component ("mucosubstances"). PA-TCH-SP positive material has also been demonstrated within the transport ductule and the mucous layer covering the specialized cuticle of the transport epithelium.

Location of the exocrine glands suggests discharge of secretory products through transport ductules into the pore channel of the coxal organ. As seen by SEM and TEM, the secretion spreads over the modified cuticle of the transport epithelium (LITTLEWOOD, 1983; ROSENBERG, 1983a) and fills up the bottom of the pore channel. Material that is electron-dense to varying degrees, interpreted as the mucous layer, has been observed in all coxal organs hitherto examined (ROSENBERG, 1985). It stains with PA-TCH-SP in *Lithobius forficatus* , but also in *Cryptops hortensis* (Scolopendromorpha; ROSENBERG, 1983b). It was suggested that this mucus consists of a hygroscopic material, which would gather water vapour from moist air (ROSENBERG, 1983a, 1985; ROSENBERG & BAJORAT, 1984).

Apart from forming the transport ductule, the role of the additional cell is unclear. Enlarged surfaces and abundant mitochondria in these cells suggest transporting ability, perhaps to modify secretion products within the ductule.

Localization of chloride in the modified cuticle of the coxal organ has been regarded as an indication of transepithelial solute transport as it is in the "chloride cells" of other transporting systems (e.g. anal papillae, anal organs, ventral tube, coxal vesicles) in a variety of insects (WICHARD & KOMNICK, 1973; KOMNICK, 1977; EISENBEIS, 1976; EISENBEIS & WICHARD, 1975). Accumulation in the mucous layer, however, seems not to be significant.

In general, the results presented here neither contradict the assumed uptake of water vapour from the air by coxal organs nor the pheromone release. However, there is still no definite proof for a hygroscopic capacity of the mucous layer; the epidermal glands close to the coxal pores are the presumed site of pheromone production (ROSENBERG, 1994).

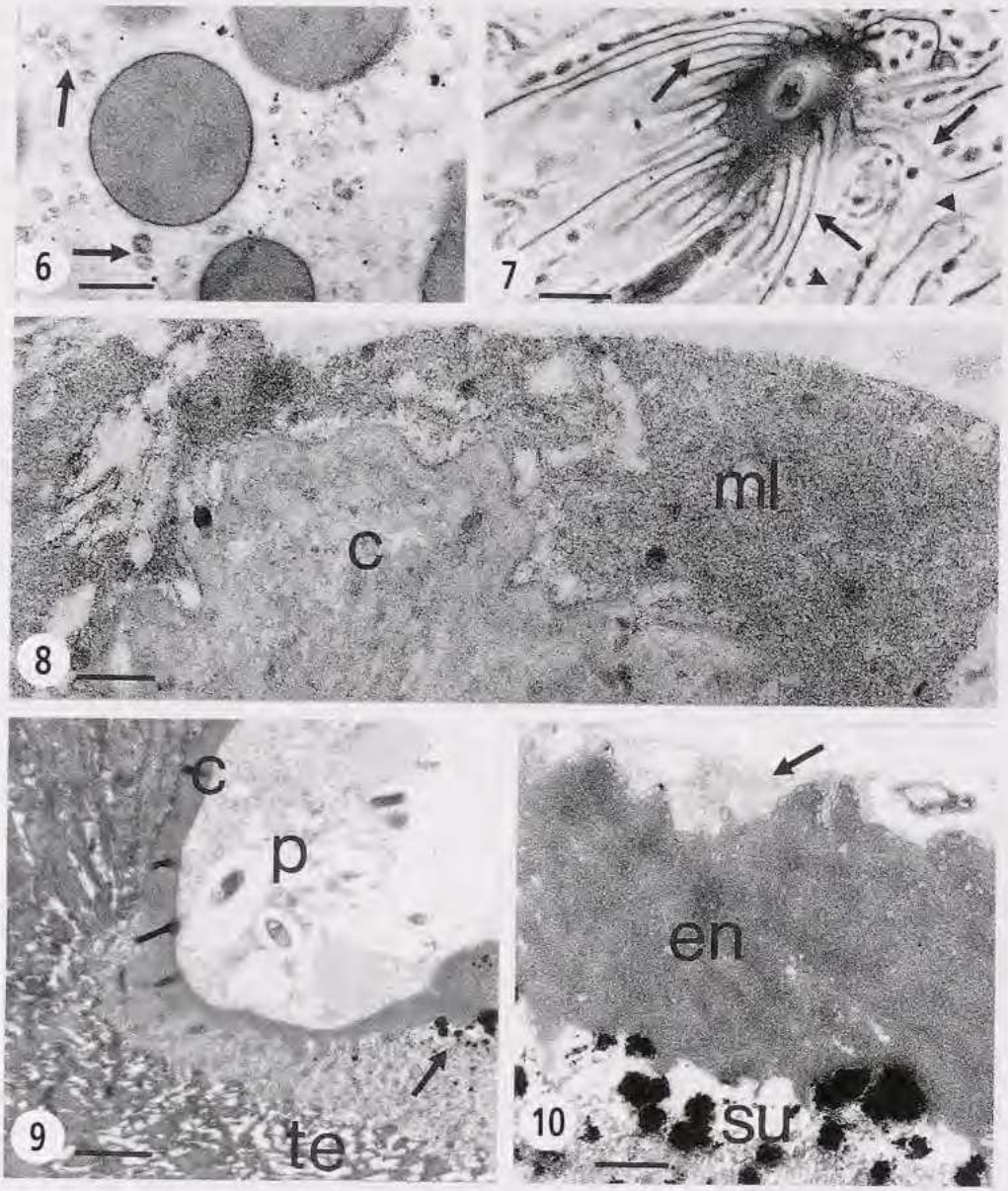


FIG. 6. — PA-TCH-SP-reaction in secretory cells (unstained sections): reaction products are visible at the margin of the secretory granules and in profiles of the ER (arrow). Scale line, 0.04 µm.

FIG. 7. — PA-TCH-SP-reaction in additional cell (unstained section): reaction products are visible within the cuticular duct (*) and the space underlying the duct, and between the microvillar infoldings (arrows). Mitochondrium (arrowhead). Scale line, 0.05 µm.

FIG. 8. — PA-TCH-SP-reaction in mucous layer (unstained section): the reaction products are only visible within the mucous layer (ml). C cuticle of the transport epithelium. Scale line, 0.03 µm.

FIG. 9. — Chloride-reaction in cuticle of the transport epithelium: a part of the coxal organ is shown with the transport epithelium (te) and the cuticle (c) of the pore channel (p). Reaction products (arrow) are only visible within the subcuticle of the main epithelium. Scale line, 0.1 µm.

FIG. 10. — Chloride-reaction in cuticle of the transport epithelium: coarse reaction products are localized within the subcuticle (su); fine precipitations are scattered within the endocuticle (en) and in the mucous layer (arrow). Scale line, 0.03 µm.

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